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Risk of bacterial foodborne pathogen infection among gastroenteritis cases in Qatar

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ABSTRACT

Foodborne illness has been determined to be one of the major limitations to the advancement of world health. Bacterial pathogens among the leading causes of foodborne illness are *Escherichia coli* (*E. coli*), *Campylobacter*, *Salmonella*, and *Listeria*. The risk of these pathogens was investigated among gastroenteritis cases in the diverse population of the state of Qatar. Fecal samples from patients admitted to Hamad Medical Corporation (HMC) with complaints of gastroenteritis were screened for the targeted pathogens using a combination of bacterial enrichments and molecular detection. *Salmonella* was the most common pathogen (42.9%), followed by *E. coli* (35.3%), and *Campylobacter* (21.0%). *C. jejuni* was the most common species of *Campylobacter* (67.4%). The probability of detection of *E. coli* decreased with age. Meanwhile, both probabilities of detection of *Campylobacter* and *Salmonella* increased with age. *Listeria monocytogenes* was much less common among gastroenteritis cases compared to the other pathogens.

1.Introduction

Foodborne illnesses is responsible for major health burdens worldwide. In the U.S. alone, it is estimated that 48 million people suffer from the foodborne diseases, 128,000 of those cases being hospitalized and 3,000 resulting in death (1).

The World Health Organization (WHO) estimates that 2.2 million people worldwide die per year of diarrhoeal food and waterborne diseases alone (2). Although data on individual countries is available, information on the global burden of foodborne diseases is lacking, but estimated cost per individual nation is high (3,4). International public health agencies such as Foodborne Diseases Burden Epidemiology Reference Group (FERG) along with WHO are currently undertaking the estimation of the worldwide burden of foodborne disease, listing *Escherichia coli* (*E.coli*), *Campylobacter* and *Salmonella spp.* among the top challenges (2). The risk of foodborne pathogen transmission is exacerbated by the ease of travel and the globalization of trade (5).

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This is especially true in international areas such as Qatar, where the ratio of expatriates to natives is 9:1. Areas of such cultural diversity are key to studying foodborne illnesses. *E. coli*, *Campylobacter* and *Salmonella* are three of the major foodborne pathogens that contribute to the burden of disease (6), especially in very international regions. We have been carrying out complementary studies on the occurrence of foodborne pathogens at different levels of the food chain, focusing on examining the presence of these pathogens at the production level among food animals, followed by products as they move through processing plants, and final products in retail stores and restaurants (7,8). This study compliments our effort in tracing and assessing the presence of these pathogens among human gastroenteritis cases. Gastroenteritis is an inflammation of the gastrointestinal tract caused by viruses, bacteria or other microorganisms. Symptoms include abdominal pain, vomiting, and diarrhoea. In addition to the immediate gastrointestinal symptoms associated with infection of these pathogens, there is the risk of chronic sequelae such as Inflammatory Bowel Disease (IBD) (9-11). In this study we assessed the prevalence and risk factors of foodborne pathogens, including *E. coli*, *Campylobacter*, *Salmonella* and *Listeria* among gastroenteritis cases in the diverse population of Qatar in hopes of shedding light on the roles of these pathogens on the condition.

2. Materials and Methods

2.1. Target and Study Populations

We carried out a cross-sectional study to address the stated objective. *E. coli*, *Campylobacter, Salmonella* spp., and *Listeria monocytogenes* were recovered from the target populations. Subjects were a subset of patients selected from individuals admitted to Hamad Medical Corporation (HMC) hospitals in Qatar with complaints of gastroenteritis during the period of August 2011 to May 2014.

Fecal samples were collected from the patients and tested bacteriologically for the presence of these pathogens. All samples were collected during routine patient care. Ethical approval was granted from the Institutional Review Board for the use of these samples and patient data. To prevent directional bias, the patients' backgrounds were diverse, including differing ethnicity, nationality, gender, age, and diagnosis. Fecal samples were obtained from a total of 1,110 patients.

2.2. Pathogen Isolation

Pathogens were isolated from human samples using standard bacteriological procedures. 1 g of the collected stool samples was diluted with 10 ml of phosphate buffered saline (PBS, pH 7.2; Sigma, St. Louis, MO, USA) and 500 µl of this dilution was added to 5 ml of Selenite broth (Oxoid, Basingstoke, Hampshire, UK) for enrichment and was incubated at 37°C for 24-48 h.

E. coli - Samples were inoculated onto sorbitol-MacConkey agar (SMAC) to isolate Shiga toxin-producing *E.coli.* From SMAC at least five non-sorbitol-fermenting (NSF) colonies, if any, were picked. All *E.coli* isolates were tested using the slide agglutination test using polyvalent and appropriate monovalent Enteropathogenic E. coli (EPEC) O- specific antiserum (Bio-Rad Laboratories, Inc., UK).

Campylobacter - Samples were incubated on Christie–Atkins–Munch-Petersen (CAMP) agar at 42°C in microaerophilic conditions for the isolation of *Campylobacter spp.* Identification of presumptive pathogens was performed using biochemical tests and serum agglutination reactions according to standard methods.

Salmonella - The enriched samples were subcultured onto MacConkey agar and incubated at 37°C for 24 h. Colonies were screened using biochemical tests such as Kilger's iron agar, motility indole-urea agar, Lysin iron agar and o-nitrophenyl-β-D-galactopyranoside. Colonies from these screenings were identified with confirmatory biochemical tests using API 20E (bioMereux, Marcy I'Etoile, France) or VITEK (bioMereux).

Listeria monocytogenes - The samples were pre-enriched with Demi-Fraser broth (Oxoid, Hampshire, England) and incubated for 22–26 h at 30°C. The enrichment broth, MOPS free acid and MOPS sodium salt (Fisher Scientific, Pittsburgh, PA, USA) was used as a selective medium in which the samples were incubated at 35°C for 18–24 h.

2.3. PCR Detection

Samples were then sent to Cornell University for further testing. Polymerase chain reaction (PCR) detection was performed on the samples to confirm pathogen presence using the BAX® Automated System (Dupont, USA). A 5 µl aliquot of the respective secondary enrichment (BHI-*Campylobacter*, *Salmonella*;EC broth –*E. coli*) was added to 200 µl of the buffer (proteinase-containing lysis buffer) provided by the manufacturer.

Samples were then heated in the lysis reagent solution to rupture the bacterial cell wall and release the DnA. PCR tablets, which contain all the reagents necessary for PCR plus fluorescent dye, were hydrated with the lysed sample and processed in the cycler/detector provided by the manufacturer. within a few hours, the PCR amplified Dgenerates a fluorescent signal, which the BAX® system application uses to analyze the findings. Results are displayed on a monitor screen as simple positive or negative symbols.

2.4. Data Collection

Data on putative risk factors associated with the presence of these pathogens were extracted from the medical records. The data included age, sex, and nationality, which was used as a proxy for food preparation. The significance of association of the presence of a particular pathogen was compared to other pathogens combined using the univariate logistic regression analysis and quantified by the odds ratio for categorical variables. Factors that were significant in the initial univariate analysis were considered further in a multivariate logistic regression analysis to assess the significance of each factor while simultaneously controlling for the association with other factors. The probability of infection, inf, with

either pathogen at a specific age was computed from the regression coefficient, where α is the constant of the respective logistic regression equation for a pathogen, β is the regression coefficient for age, and Xi was a specific age for the patient.

$$P(inf) = \frac{1}{1 + exp - (\alpha + \beta_i(age))}$$

β_i is the logistic regression coefficient for age and interpreted as the changes in the log odds of infection with the respective pathogens due to one-unit change in age.

3. Results

The 1110 patients sampled were admitted with the complaint of gastroenteritis and had stools obtained and examined for the targeted pathogens. Patients admitted with the complaint of gastroenteritis, but who had no stool samples collected, were excluded from the study. Among patients that met the inclusion criteria, 476 tested positive for *Salmonella* (42.9%), 392 for *E. coli* (35.3%), 233 for *Campylobacter* (21.0%) and 9 for Listeria (0.8%). Overall, among the four pathogens screened for, *Salmonella spp.* was the most common pathogen detected amongst patients. Consequently, it was about twice more likely to detect *Salmonella spp.* among gastroenteritis patients compared to *Campylobacter spp.* *E. coli* samples were tested for pathotype lineages 1, 2, 3 and 4 using serotyping. EPEC 4 were detected in 123 of the samples while EPEC 2 and 3 were detected in 57 and 98 of the samples, respectively.

Pathotype 4 was significantly more common among cases with *E. coli* compared to pathotypes 2 or 3 (P < 0.05). However, there was no significant difference in the occurrence of pathotype 2 or 3 among the isolates. *Campylobacter* samples were further tested for species. More than two thirds (155) of the isolates were genotyped as *C. jejuni* (66.5%), 42 were *C. coli* (18%), 2 had both *C. jejuni* and *C.coli* (0.9%). Only one sample was identified *C. upsaliensis* and 33 samples were undetermined (14%). *Salmonella* was further tested for groups; most samples belonged to Group D (35.5%) and Group B (31.1%). The other subgroups (A, C, C1, C2, G, and G1) were detected among the study population, but at significantly lower proportions (Table 1).

Table 1. The distribution of *Salmonella* group among the gastroenteritis patients enrolled in the study.

Salmonella Group	n	Prevalence (%)
A	3	0.6%
B	148	31.1%
C	11	2.3%
C1	29	6.1%
C2	12	2.5%
D	169	35.5%
E	15	3.2%
G	1	0.2%
G1	3	0.6%
Unknown	85	17.9%

admitted during the hot (April-Nov.) or cold (Dec.-March) season of the year. Our data showed that *E. coli*

Table 2. The distribution of virulence genes, serotypes, and pathotypes of *E. coli* among patients enrolled in the study.

Virulence genes and serotypes	Total Samples Tested	Total Positive Samples	Prevalence (%)
STEC			
stx	311	18	5.8%
eae	311	72	23.2%
Both	311	10	3.2%
Other genes			
F17	96	1	1.0%
F41	96	75	78.1%
Serotypes			
O157:H7	266	7	2.6%
O26	78	1	1.3%
O111	78	7	9.0%
O121	78	0	0.0%
O45	78	1	1.3%
O103	78	0	0
O145	78	0	0
Pathotypes			
EPEC 2	392	57	14.5%
EPEC 3	392	98	25.0%
EPEC 4	392	123	31.4%
Unknown EPEC	392	39	9.9%
Unknown	392	75	19.1%

Subsets of the *E. coli* samples were also further tested for the virulence genes stx and eae, as well as STEC serotypes. A total of 311 *E. coli* isolate samples were further tested for the STEC virulence genes. The stx gene was detected in 5.8%, the eae was detected in 23.2%, and both genes were detected in 3.2% of the samples. A subset of 266 samples were tested for O157, only 7 were positive (2.6%). Samples that were positive for either stx or eae were further evaluated for the six main food adulterants non-O157 STEC serotypes: O26, O45, O103, O111, O121 and O145. Serotype O111 had the highest occurrence among these samples (9%), and 1.3% were positive for O26 and O45 (Table 2). None of the other serotypes were detected among the samples. In addition, a total of 96 samples were tested for the F17 and F41 genes. Overall, 78.1% were positive for F41 and only 1% were positive for F17 gene. Evidently the F41 gene was more common among the samples. recovered from humans (Table 2). Next, we examined the host (patients') factors that would predispose them to the risk of gastroenteritis, these included age, sex, the region they were from, and whether they were

was common among younger patients with an average age of 1.2 years (median = 0.9 years, range (0.1 to 68 years)), whereas for *Campylobacter* it was 7.7 years (oldest: 64, youngest: 2 months). As for *Salmonella,* it was 10.3 years (oldest: 86, youngest: 1 month and 10 days).

Over half of the patients admitted with *E. coli* and *Campylobacter* were men (51.4%; 57.3%) but of those admitted with *Salmonella*, 60.7% were women. Furthermore, there was a relationship between the season of admission and the likelihood of the pathogens (Figure 2). It was 5-times more likely to admit a patient with an *E. coli* infection in the colder months of the year in comparison to the hotter months (Odds ratio (OR) = 5.0 and the 95% confidence interval (CI) = 2.7, 9.0). Similarly, patients diagnosed with *Campylobacter* infection were twice more likely to be admitted in the colder months in comparison to the hotter months of the year (OR= 2.6, 95% CI = 1.5, 4.5).

The situation with *Salmonella* was inverse, it was less likely to admit patients with the infection during to the colder months in comparison to the hotter months OR= 0.2, 95% , CI=0.1, 0.4). Patients were from a total of 33 countries which were then grouped into regional areas with the perception that patients from the similar regions had similar social behaviors. The majority of patients were from the Middle East, including Qatar, followed by Asia (3). Africa, and European origin, including the USA (Table Occurrence of the pathogens among patients from different regions was similar, though the number of patients with Salmonella from Asia was notably higher than *E. coli* or *Campylobacter*. Patients from

the Middle East had a lower prevalence of *Salmonella* than the other two pathogens, and the patients from Africa had a lower prevalence of *E. coli* than the other two pathogens (Table 3).

Table 3. The distribution of patients enrolled in the study by pathogen and region (count and percentage).

Source	E. coli	Campylobacter	Salmonella
Middle East	210 (81.1%)	74 (77.9%)	92 (71.9%)
Asia	31 (12.0%)	11 (11.6%)	23 (18.0%)
Africa	12 (4.6%)	6 (6.3%)	9 (7.0%)
Europe	6 (2.3%)	4 (4.3%)	4 (3.1%)

The odds of the risk of gastroenteritis, by pathogen, were evaluated for significance of association with age, region, and sex using the logistic regression analysis and quantified using the OR. The only statistically significant factor was age (Table 4). This data was used to calculate the probability of infection by age for the three targeted pathogens (Figure 3).The analysis showed that the risk of infection with E. coli decreased significantly at a rate of -0.31 in the log-odds per year increase in age (Figure 3). The probability of infection when age was less than one year old was 63% (as calculated from the logistic regression, calculated when age is set to zero value). However, this probability decreases as the patient gets older and approaches zero when the patient's age approaches 20 years.

Table 4. The results of the logistic regression analysis for each pathogen and the age of the patient among the study population.

Category		Regression coefficient	Standard error	p-value
E. coli	Age	-0.329	0.07	*0.000*
	Constant	0.814	0.14	*0.000*
Campylobacter				
	Age	0.03	0.01	*0.004*
	Constant	-1.70	0.14	*0.000*
Salmonella				
	Age	0.04	0.01	*0.000*
	Constant	-1.18	0.12	*0.000*

On the other hand, the probability of infection with either *Campylobacter* or *Salmonella spp.* on average and irrespective to age (age constant at less than one year), were 15 and 23%, respectively (Figure 3). These probabilities increased with age at a relatively lower rate for *Campylobacter* in comparison to *Salmonella* spp. (Figure 3). Due to the low prevalence of *Listeria*, it was not included in the logistic regression, though it should be noted that all cases occurred during the hot season.

4. Discussion

This study is part of our long-term objective of developing risk assessment for foodborne pathogens in different populations. The focus was on four of the main pathogens that burden health systems around the world: *E. coli*, *Campylobacter*, *Salmonella* and *Listeria*. Accurate data on the burden of foodborne diseases are lacking however, though partial estimates from different industrialized countries indicate a high burden to the health systems. The WHO highlighted a report in 2005 which indicates 1.8 million people around the world died from diarrhoeal diseases and

emphasizes the importance of epidemiological data in these estimates. In the USA, it was estimated that 48 million individuals suffer annually from these illnesses with direct cost of disease ranging between $4.4 and $144.6 billion (1). Similar high cost estimates have been obtained from other countries, including the Netherlands and other parts of the world (1-4). In this study we assessed the prevalence and risk factors of *E. coli, Campylobacter, Salmonella,* and *Listeria* among gastroenteritis cases in the diverse population of Qatar in hopes of shedding light on the roles of these pathogens on the condition. We used a multidisciplinary approach and a hybrid design epidemiological study to investigate the occurrence of these foodborne pathogens among gastroenteritis cases (12). This design has the advantage of combining elements of cross-sectional studies to determine the starting point and longitudinal component designs to integrate follow-up information.

Qatar was chosen due to its diverse social and cultural population which also includes diverse food and methods of food preparation. Because of this diversity, we would expect to see diverse pathogens associated with the risk of gastroenteritis. More knowledge gained on the mechanisms of disease in a diverse population would greatly aid in mitigating the risk of these pathogens. Estimates of foodborne illness are difficult to make due to the number of potential incriminated pathogens and the fact that not all cases are tested for specific pathogens.

A survey in the US from 2000-2008 determined that the number one cause for hospitalization was *Salmonella* (35% of cases) followed by *Norovirus* (26%) and *Campylobacter* (15%) (13). Our findings were similar, *Salmonella* was most common in hospitalized

patients, however, we did not screen for viruses. Earlier studies showed that in the US, *Salmonella* serogroup B was common in the mid-90s but declined over the years, while in Europe serogroup D was common but declined over the years (14). In our study, serogroup D was the most common followed by serogroup B. The differences of the proportion of serogroups in the previous studies and ours could be attributed to difference in the population and differences in time. In Saudi Arabia, a neighboring country with similar cultural habits, serogroups D and C are more prevalent than serogroup B (14). This observation lends credence to the variation in the occurrence of these serogroups among different populations and geographical areas. One of our earlier studies showed a similar prevalence of *Campylobacter* (23%) in human fecal samples taken from patients complaining of gastroenteritis in Qatar, second to *Salmonella* at 52% (7). However, the European Centre for Disease Prevention and Control reported in 2015 that *Campylobacter* was the most commonly reported foodborne pathogen in the European Union, followed by *Salmonella* (15). Similar to our study, *C. jejuni* and *C. coli* are shown to be the leading species causing gastrointestinal distress worldwide, but other species, such as *C. concisus* are of emerging concern (16). *E. coli* is among the most studied bacterial pathogen recovered from animals, humans, and the environment. Varying diagnostic schemes are employed by different laboratories and hospitals around the world. Adding to the complexity of the issue is the differing identification methods of the virulence genes in *E. coli* among different populations. While the traditional methods used in grouping *E. coli*

into pathotypes have aided in tracking outbreaks and sources of the pathogens, continuous evolvement of these methods makes it difficult for results to be comparable (17). We have focused on a subset of *E. coli* factors in hopes of making the data comparable to others and in order to compare the results to the findings in animals and environmental sources along the food supply chain in the same population (8,18). Gastroenteritis associated with *E. coli* infections in humans is a complex and perplexing issue due to the presence of different factors, including hosts and pathogenicity. Host susceptibility is a major factor in addition to *E. coli* canalization, virulence, and pathogenicity (19). In our investigation, among the agent's factors, we focused on the Shiga toxin-producing *E. coli* (STEC) stx gene, the attaching-and-effacing gene (eae), virulence genes (e.g., F17 and F41), and serotypes that have been associated with *E. coli* outbreaks of food poisoning. Most clinical laboratories around the world have attempted to shed light on the mechanisms by which organisms play a role in gastroenteritis and have examined the pathogens for the presence of STEC as part of the pathotype spectrum (17).

The main explanation for this approach is that STEC have been associated with human illness (20). Studies that investigated *E. coli* strains recovered from patients with gastroenteritis have adopted different approaches which include the pathotype and serotype. Many studies report results similar to ours where STEC was significantly less common among cases relative to Enteroaggregative *E. coli* (EAEC) (20-23). The differences among these cases depended on the population from which the samples were recovered and the purpose of submitting the samples.

Adding to the complexity of the issues are the recommendations of the Center for Disease Control and Prevention (CDC) regarding testing for the STEC, which are not applied uniformly by laboratories around the world (24).

The data on the different *E. coli* serotypes isolated from patients clinically diagnosed with gastroenteritis are scarce. However, available data are biased towards focusing on the O157:H7, which has been the main target in numerous investigations (25-27). In our study, we examined both O157:H7 and non-O157 serotypes and O111 was the most common. A study in Brazil had similar results regarding the high occurrence of O111 among patients with diarrhoea (21). This was not the case among other studies around the globe and the differences could be attributed to the difference between the populations, the number of samples tested, and the detection method being used.

In addition, the interpretation of the term gastroenteritis varies by different clinicians and hospitals, which might create confusion regarding the association between the syndrome and the pathogen isolated. Inflammation of the stomach and the intestine could result from different etiologic factors that were unlikely to have been investigated at the time of the stool collection (28). Another factor that led to confusion in the literature was the use of causative agent versus association. Sometimes when a pathogen is isolated from patients clinically diagnosed with gastroenteritis there is a tendency among health professionals to declare that that agent is the "sole" etiologic factor, rather than concluding it associated

with the condition; many other factors could have been present at the time but are not investigated. In regards to patient age, *E. coli* is known to mostly affect younger children, which is concurrent with our findings that age is a statistically significant risk factor. In our study the majority of all patients were under 10 years of age, but there were more older patients with *Campylobacter* and *Salmonella*. A study from Uruguay reported that *E. coli* was the only bacterial pathogen that was isolated from children under 5 years old diagnosed with acute gastroenteritis (29). Our study targeted all ages diagnosed with foodborne bacterial pathogens and showed that the probability of pathogen detection decreases with age. We believe that the observations regarding the association between the country/region of origin and the risk of gastroenteritis were not conclusive. Many factors could have played role in the observed results including the socioeconomic classes, overlap in the food preparation among people from different geographic region, health awareness, and access to health services. Initially we thought that the food preparation and handling could contribute to the risk of gastroenteritis as reported in other studies (30,31). Although there were differences in our data regarding the association between the presence of the targeted foodborne pathogens and the geographic origin of the patients, we believe that the results are inclusive, as was reported in another study due to some of the factors listed above (32). The foodborne pathogens investigated in this study are among the common pathogens that pose significant burden of disease around the world (1,2,5). Gastroenteritis infections caused by these pathogens are mostly self-limiting and admissions to hospitals

are not common. In a few cases, around 6-7%, patients with gastrointestinal illness may develop sequelae with serious consequences such as inflammatory bowel disease (IBD) (4). Studies on drawing from medical records of 2,000 general practitioners in the UK looked at the occurrence of IBD (including Crohn's, ulcerative colitis and indeterminate colitis) in patients admitted with acute gastroenteritis. The estimated incidence rate of IBD for patients admitted with gastroenteritis was 68.4 per 100,000 person-years vs 29.7 per 100,000 person-years in the control group (9). A study in Denmark, looking at the short and long-term (over a 15 year – period) risk of IBD in patients with prior history of *Salmonella* and *Campylobacter* gastroenteritis, found that the greatest risk of IBD was in the first year after infection (10). Another study in Sweden showed similar results (33). In order to shed more light on this issue, for future studies we would like to follow up on the patients in this study to see if they have been diagnosed with any sequelae such as IBD.

5. Conclusion

The association between age and specific pathogens observed in our studies is similar to other reports from different parts of the globe (22,23,29). *E. coli* is known to mostly affect younger children, which is concurrent with our findings that age is a statistically significant risk factor. In this study the majority of patients with *Campylobacter* and *Salmonella* were under 10 years of age, but there were also older patients. These findings were consistent in another report which indicated that both pathogens are associated with all age groups. It should be noted that we only tested samples from patients with clinical diagnosis of gastroenteritis.

Furthermore, the use of the term gastroenteritis is broad in the literature and most of the etiological agents, including viruses, present similar symptoms or in some incidences no symptoms (34). However, the focus of our study was on foodborne bacterial pathogens.

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